

STIC-ILL

From: Shibuya, Mark
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Subject: Reference request for 09/403,539 Dean et al. Oral bioavailability

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Please send the following:

Pharmaceutical research (UNITED STATES) Apr 1998, 15 (4) p583-91, ISSN 0724-8741

Biochemical pharmacology (ENGLAND) Aug 8 1995, 50 (4) p571-6, ISSN 0006-2952

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Thank you,

Mark Shibuya
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S354 Thursday 16 September 1999

NY-ESO-1 Ab was detected: in 1 pt after curative tumor resection, in 3 pts with PR of metastatic disease under therapy, and in 1 pt with a NY-ESO-1 - tumor relapse. Our results suggest that NY-ESO-1 Ab is dependent on the presence of NY-ESO-1 + tumors, reflecting the evolution of disease.

1433

ORAL

Clinical and immune responses in metastatic melanoma patients immunized with an anti-idiotypic (anti-Id) monoclonal antibody (mAb) mimicking disialoganglioside gd21

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Purpose: We initiated a clinical trial for patients with metastatic melanoma treated with an anti-Id mAb (TriGem) that mimics GD2. The primary goal was to determine immune responses & toxicity; secondary goals were clinical responses & survival.

Patients & Methods: Forty-seven patients received either 1, 2, 4 or 8 mg doses of TriGem mixed with 100 µg of QS-21 adjuvant s.c. weekly x 4 then monthly until disease progression.

Results: Hyperimmune sera from 40 of 47 patients revealed an anti-Id (Ab3) response. The 7 who did not generate an immune response progressed on study prior to the fifth injection. Patient Ab3 was truly Ab1 since it specifically bound to purified GD2. The Ab3 was predominantly IgG, with all IgG subclasses represented. One patient had a complete response, 17 patients are stable on study & 27 progressed and 20 have died. The Kaplan-Meier derived overall median survival has not been reached but at 18 months was 52%. Toxicity consisted of local reaction at the site of the injection & mild fever & chills.

Conclusion: TriGem has minimal toxicity, generates strong & specific IgG immune responses against GD2, & appears to have a major favorable impact on disease progression & survival.

1434

ORAL

Gene therapy for colon cancer using a novel deoxythidine kinase suicide gene together with cytosine arabinoside

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Purpose: Cytosine arabinoside (ara-C) is an effective drug for treatment of acute myeloid leukemia. However, it has only a limited validity against most GI tract tumors such as colon cancers. The reason includes relatively less amounts of deoxycytidine kinase (dCK) activity in non-myeloid cells. Here we hypothesized that solid tumors could be sensitized to ara-C if we successfully increased the cellular dCK activity. To verify the assumption, we transduced the dCK cDNA to mouse colon cancer cells and examined the efficacy of ara-C.

Methods: The MC38 mouse colon carcinoma cells were retrovirally transduced with dck cDNA. After selected with geneticin, expressors (MC38-dck) were isolated and compared to the wild-type (MC38-wt) or control cells (MC38-neo). In order to examine the sensitivity, we performed cytotoxic assays. Next, we constructed an adenoviral vector containing dCK cDNA under the CMV promoter. Using this vector, we evaluated the in vivo efficacy of the dCK.

Results: Compared to other cells, MC38-dck cells were significantly sensitive to ara-C (IC50 = 488 pM). While MC38 cells transduced by control Ad.CMV-b-gal did not demonstrate differences in a sensitivity to ara-C, cells infected by Ad.CMV-dck exhibited an MOI-dependent increase of the sensitivity (MOI, 0 = 23.4 nM, MOI, 100 = 5.86 nM, MOI, 500 = 1.95 nM, respectively). When implanted subdermally, tumors of dCK-transduced MC38 cells were significantly smaller than of non-transduced cells after treatment of ara-C (p < 0.05, two different measurements).

Conclusion: This system has numerous advantages for non-myeloid cancer gene therapy. First, since the dCK cDNA is human origin, it potentially limits the immunological responses. Next, the working concentration of the produg demonstrated in this study is easily achievable in the patients. Lastly, since ara-C is a classic agent, its pharmacokinetics is fully understood. Taken together, the dck/ara-C suicide system may be a potent approach for gene therapy of colon cancers.

1435

ORAL

Phase I-Study for patients with inoperable pancreatic carcinoma with encapsulated cells producing cytochrome P450 CYP2B1 that activates ifosfamide

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Background: Conventional chemotherapy of pancreatic carcinoma is only marginal effective. Substances such as ifosfamide, registered for the treatment of pancreatic cancer, have not been followed up due to a high toxicity at therapeutic doses.

Hypothesis: The local conversion of ifosfamide into its active components, phosphomustard and acrolein, should be feasible for treatment employing low systemic concentration of the drug.

Rationale: Transfection of CYP2B1 in cells with subsequent microencapsulation.

Experimental Work: The enzyme activity (resorufin-assay) remains stable for weeks in vitro and in vivo within the microencapsulated CYP 2B1-expressing cells. We could demonstrate a significant antitumorous effect of the intratumorally injected capsules on xenotransplanted human pancreatic carcinomas on the nude mouse (Gene Therapy 1998, 5: 1070-1078). Angiographic experiments in pig assured the feasibility of an intraarterial placement of the capsules into the pancreas (Ann NY Acad Sci 1999, in press). A clinical protocol was established and approved (J Mol Med 1999, 77: in press).

Patients, Material and Methods: L293-cells were transfected with CYP2B1-gene, microencapsulated (diameter 0.5 cm) under GCP-conditions and packed sterile. Patients with confirmed inoperable adenocarcinoma of the pancreas underwent angiography and capsules were injected into a vessel leading to the tumor. The patients were monitored for 48 hrs to exclude allergic reactions or pancreatitis. A day later, ifosfamide was administered at 1000 mg/m² BS for three consecutive days to be repeated day 21-23. The patients were followed-up for 6 months.

Results: The study was opened 7/98. A total of 12 patients were enrolled; 5 of them finished the entire study period. In 10/12 patients the capsules could be administered as planned. In one patient, this was technically impossible. Another patient experienced an acute abdomen and had to be operated for an ileus. The 10 patients treated tolerated the procedure without any complications. No allergic reactions or pancreatitis was encountered. Chemotherapy was uneventful.

Discussion: The intraarterial application of microcapsules for targeted chemotherapy was well tolerated. The antitumoral effects cannot be judged at present time.

1436

ORAL

Oral administration of chimeric MBO antisense-Protein Kinase A inhibits growth, angiogenesis and growth factors production and cooperates with cytotoxic drugs in human cancer xenografts

G. Tortora¹, V. Damiano¹, R. Bianco¹, G. Fontanini², R. Caputo¹, S. De Placido¹, A.R. Bianco¹, S. Agrawal³, F. Ciardiello¹. ¹Oncologia Medica, Università Federico II, Napoli; ²Anatomia Patologica, Università di Pisa, Pisa, Italy; ³Hybridon, Inc., Millford, MA, United States

Introduction: Protein kinase A type I (PKA) plays a key role in neoplastic transformation and conveys mitogenic signals from different growth factors and oncogenes. Different pharmacologic tools developed to inhibit PKA expression and function are able to inhibit cancer cell growth in vitro and in vivo. We have recently shown that a novel class of mixed-backbone oligonucleotides (MBOs) targeting the PKA subunit RI, exhibit improved pharmacokinetic properties and antitumor activity in vitro and in vivo in several human cancer types.

Methods: We have administered orally HYB 165, a chimeric DNA/RNA MBO targeting the PKA, alone and in combination with different cytotoxic drugs. We have evaluated the tissue distribution, the pharmacokinetic and the effect on tumor growth, angiogenesis and expression of several factors involved in the control of cell proliferation.

Results: We have demonstrated that the chimeric MBO HYB 165 has a good bioavailability and accumulates as intact oligo in the tumor after oral administration. As compared to a scramble MBO, oral HYB165 demonstrated a dose-dependent inhibition of the growth in human cancer xenografts in nude mice. Histochemical analysis showed that the antitu-

mor effect was accompanied by inhibition of expression of different growth factors of the EGF family, inhibition of angiogenesis and increase in p27 expression. Moreover, when oral HYB 165 was used in combination with cytotoxic drugs, such as taxanes and platinum derivatives, a marked cooperative effect was observed resulting in sustained inhibition of tumor growth, growth factor production and angiogenesis and in a significant increase of the survival of treated mice.

Conclusions: This study is the first demonstration of the antitumor activity of an antisense oligo after oral administration. Moreover, the inhibition of expression of factors involved in the control of cell proliferation, cell cycle and angiogenesis by this novel chimeric MBO antisense PKAI represents the rationale for the future use of HYB 165, which has recently completed a phase I trial in cancer patients by i.v. route with an excellent toxicity profile.

1437

POSTER DISCUSSION

IkB-gene therapy does not enhance chemotherapy-induced apoptosis in HCC

M.K. Tietze, S. Kubicka, C. Trautwein, T. Wuestefeld, J. Plümpe, M.P. Manns. *Dept. of Gastroenterology, Medizinische Hochschule Hannover, Germany*

Hepatocellular carcinomas are resistant to systemic chemotherapy. The effect of chemotherapeutic agents is mainly mediated by apoptosis. Since there is evidence that inhibition of NFkB might dramatically increase the susceptibility of tumor cells to TNF- and chemotherapy-induced apoptosis, we investigated the Adenovirus-IkB superrepressor as an option for multimodal gene therapy for HCC.

Methods: We studied three different hepatoma cell lines: HepG2, Huh7 and a primary, early, passage HCC-cell line (Tu 5), we established from a human HCC. Apoptosis was quantified by FACS analysis. NFkB activation was investigated by gel shift experiments. For in vivo investigations xenotransplants in nude mice were transduced with Adenovirus-IkB and subsequently the mice were treated by i.p. doxorubicin (ADM) (7.5 mg/kg).

Results: In all cell lines a chemotherapy- and TNF-mediated upregulation of NFkB could be demonstrated. In cells treated simultaneously with ADM and TNF NFkB expression was significantly enhanced. Although transduction with IkB-adenovirus completely inhibited NFkB activation, there was no increase of apoptosis in cells treated with ADM and IkB. In contrast apoptosis was dramatically increased by the combination of TNF and IkB-adenovirus. Also, we could demonstrate an enhancement of apoptosis in cells treated with TNF and ADM. TNF and ADM mediated apoptosis was efficiently inhibited by dnFADD. These results could be confirmed by in vivo experiments.

Conclusion: Although ADM, as well as TNF, activate NFkB and death receptor pathways via FADD, ADM mediated apoptosis is independent of NFkB inhibition in contrast to TNF induced apoptosis.

1438

POSTER DISCUSSION

A high correlation between mutant p53 and increased expression of vascular endothelial growth factor (VEGF), combining those markers indicates poor effect by adjuvant tamoxifen in ER positive patients

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Purpose: To determine the possible association between VEGF content and p53 status, including analysis of mutation types and locations, in a series of 224 primary breast carcinomas, and the potential prognostic value of those markers.

Methods: VEGF was measured in cytosols with an ELISA, p53 status was determined with cDNA-based sequencing and IHC using the antibody Pab 1801.

Results: p53 mutations by sequence-data was found in 37 (16.5%). p53 positivity by IHC was found in 39 (17.4%), the median value of VEGF was 256 pg/mg. Significant associations were seen between higher VEGF content and both mutant p53 ($p = 0.0019$), and p53 IHC positivity ($p = 0.0068$). Deletions, insertions and stop codon had significantly higher VEGF values ($p = 0.0043$) than point mutations or wt p53. Mutant p53 was correlated to a worse outcome; RFS ($p = 0.0519$), BCSS ($p = 0.0310$). VEGF was correlated to outcome in ER positive patients receiving adjuvant tamoxifen RFS ($p = 0.0471$), BCSS ($p = 0.0064$). Combining those markers was of prognostic value in all patients, RFS ($p = 0.0377$), BCSS ($p = 0.0292$).

and in patients that received adjuvant tamoxifen, RFS ($p = 0.0488$), BCSS ($p = 0.0342$).

Conclusions: The results indicates that VEGF expression seems dependent on wild-type p53 loss. Combining those markers indicates poor outcome, also in ER positive patients treated with adjuvant tamoxifen.

1439

POSTER DISCUSSION

Optimisation of cervical tumour lysate loading of dendritic cells (DC) for development of a versatile and efficient anti-cervical cancer vaccine: Use of Caski cells as a model source of cervical cancer derived tumour lysate

M. Adams¹, H. Navabi¹, B. Jasani², C. Lipetz³, A. Evans³, M. Mason¹. ¹Velindre NHS Trust, Oncology, Cardiff; ²University of Wales College of Medicine, Pathology, Cardiff; ³University of Wales College of Medicine, Gynaecology, Cardiff, United Kingdom

Rationale: Murine DC loaded with HPV 16 E7 peptide can prime powerful peptide specific CTL in vivo anti-tumour response against HPV 16 E7 bearing tumours (1). Inadequate representation of tumour antigens and haplotype restriction of synthetic peptides have limited their clinical application. However, DC loaded with tumour lysates has proved an effective alternative in treatment of melanoma (2). Nevertheless, due to paucity of cervical cancer tissue available for tumour lysate preparation, it has proved necessary to improve the efficiency of antigen loading of DC prior to clinical trial.

Aim of Study: To determine the effect of ultra-sonication and/or liposome incorporation of tumour lysate on efficiency of antigen loading of human peripheral blood derived DC, using Caski cells as a surrogate source of HPV 16 E6 & E7 antigen positive cervical cancer tissue.

Method: DC generated from human volunteers' peripheral blood derived monocytes cultured for 7 days with GM-CSF and IL-4 (3), were loaded with Caski cell lysate prepared by repeated freezing-thawing \pm ultra-sonication and/or exposure to cationic liposomes DOTAP (4). Antigen loaded DC were irradiated, co-cultured with autologous PBMCs, and after two stimulations, the CTL activity was measured in a standard ⁵¹Cr release assay. HPV 16 E6 & E7 specific HLA restricted CTL activity induced was estimated using autologous BLCL targets transfected with recVac-HPV (TA-HPV) as antigen specific positive control, Wyeth-Vac as antigen negative control, and recVac-HPV (TA-HPV) as allogeneic HLA negative control.

Results: A significant HLA restricted CTL response ($>10\%$ ⁵¹Cr release at 2 E:T ratios) was demonstrated only by PBMCs primed with DC loaded with either ultra-sonicated and/or liposome exposed Caski cell lysates, at Caski cell: DC ratio as low as 1:1, indicating a need for as little as 12 mm³ of cancer tissue per 6 dose treatment regime.

Conclusion: The study supports tumour lysate as an adequate source of antigen for loading of DC for broad spectrum haplotype unrestricted priming of anti-cervical cancer specific CTL responses in a clinical trial setting.

[1] Mayordona et al. *Nature Med* 1995; 1: 1297.

[2] Nestle et al. *Nature Med* 1998; 4: 328.

[3] Romani et al. *J Exp Med* 1994; 180: 83.

[4] Nair et al. *Int J Cancer* 1997; 70: 706.

1440

POSTER DISCUSSION

Histamine dihydrochloride (Maxamine™), interleukin-2 (IL-2) and interferon- α (IFN- α) in multiple myeloma

U.-H. Mellqvist, E. Wallhult, K. Hellstrand, B. Nilsson, M. Bruna. *Dept. of Medicine, Sahlgrenska University Hospital, SE-413 45 Gothenburg, Sweden*

Background: Treatment with IL-2 and Maxamine has shown promise in melanoma and AML. Maxamine reverses the anergy and apoptosis of NK cells and T cells after their exposure to monocyte-derived reactive oxygen metabolites *in vitro*. Thereby Maxamine, acting via H₂ receptors on monocytes, synergizes with the activating effect of IL-2 and IFN- α on both T- and NK-cells.

Methods: Seven male patients, median age 50 years (45-63) were enrolled at a mean of 17 months (10-64) after PBSC. Three patients, one in CR and two in PR at inclusion, were undergoing IFN- α treatment. Four other patients had relapsed and 3 had progressive disease (PD), whereas one had stable disease (SD) after salvage therapy. IL-2 (50 μ g, bid, s.c.) and Maxamine (0.5 mg, bid, s.c.) were self-administered by all 7 patients in repeated courses of 21 days until progression or for 6 mo. The three PR and CR patients also continued on IFN- α (9 mIU/week). Follow up: (from start of Maxamine therapy). The CR patient remains in CR 33+ mo at last evaluation. One PR patient is now in CR at 39+ mo. And the other

administered at 1000 mgm for three consecutive days, TO 17033084512
day 21-23. The patients were followed-up for 6 months.

Results: The study was opened 7/98. A total of 12 patients were enrolled; 5 of them finished the entire study period. In 10/12 patients the capsules could be administered as planned. In one patient, this was technically impossible. Another patient experienced an acute abdomen and had to be operated for an ileus. The 10 patients treated tolerated the procedure without any complications. No allergic reactions or pancreatitis was encountered. Chemotherapy was uneventful.

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Biotherapy-gene therapy-vaccination

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